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Orchard establishment, precocity, and eco-physiological traits of several pomegranate cultivars

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ABSTRACT

California faces many threats to food security, ranging from water limitations resulting from long-term drought to invasive pests and diseases. Major tree crops, such as citrus and avocado, are threatened by Citrus Greening and Fusarium Dieback, respectively, posing significant economic losses to growers and farm sustainability. Pomegranate (*Punica granatum* L.) was previously a minor tree crop in California, but has become an important specialty crop, with planted area increased by 10-fold during the last twenty years, and is currently a $200 million annual industry. Pomegranate is not threatened so far by any pest or disease and is a drought- and salt-tolerant crop that can be cultivated on marginal land, which makes it an attractive alternative crop for the growers facing water and disease issues. For this investigation, two pomegranate field trials were initiated and followed over four years to evaluate site effects on establishment, precocity, photosynthesis and water relations to assist in determining appropriate cultivars for coastal versus inland climates. Traits measured included orchard establishment, photosynthesis, water potential, and flowering and yield traits. There were significant site and cultivar effects on many traits as well as site-cultivar interactions. The coastal trial grew significantly faster than the semi-arid inland site, however, the inland site was more productive than the coastal site for the first three years. Production during year four of establishment was similar at both sites.

1. Introduction

Global food security is threatened by climate change, which includes increasing temperatures and other unpredictable changes in weather patterns potentially damaging to agricultural systems (Altieri and Nicholls, 2017). For example, long-term drought in California has caused significant economic losses to farmers (Medellín-Azuara et al., 2016), with extreme and exceptional drought affecting significant percentages of California land from 2013 until early 2017. Drought in California has driven farmers to engage in crop abandonment, stress irrigation, the replacement of water-intensive crops with alternative crops (Medellín-Azuara et al., 2016), and most recently, using secondary water resources often available from urban or suburban wastewater systems. To lessen the impacts of climate change, it has been proposed that crop diversification may reduce the risk of food system vulnerability (Altieri and Nicholls, 2017).

Challenges posed by drought and water scarcity issues have led physiologists and breeders to focus on water-use efficiency in agriculture (Wallace, 2000). Improving production efficiency and drought tolerance through cultivar selection has been proposed in citrus (Savé et al., 1995) *Prunus* species (Rieger and Duemmel, 1992), dates (Djibril et al., 2005), and coffee (DaMatta, 2004). Because tree crops have some degree of variability of physiological traits among genotypes in the same species, it is useful to study diversity within crop species to determine if there are cultivars that use water more efficiently or are as productive in cropping system conditions of high abiotic stress. Pomegranate is touted as a drought tolerant crop, especially once established (Stover and Mercure, 2007), and it is being evaluated in California as an alternative tree crop species to replace or supplement more water-intensive species, such as avocado, citrus and almond (McClurg, 2015), all three of which face pest and disease threats of their own.

Historically, pomegranate has been growing in California for hundreds of years, even before statehood, starting with the Spanish missionaries who arrived in the 1700s (Day and Wilkins, 2009) and allegedly planted mongrel seed from Spain in their gardens. Pomegranate is a minor crop in California with a market value of approximately $200 million annually for juice and fresh market. The body of knowledge regarding differences in pomegranate physiology, establishment, and

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site/climate effects among cultivars conserved in germplasm is limited. Even less is known about how climate affects precocity, the reproductive biology, flowering, and fruiting of young pomegranate trees. This limitation is a barrier to commercial growers and nurseries adopting new or flouted cultivars that have traits with the potential to decrease water use and increase consumer demand for the fruit. Breeders also benefit from having germplasm phenotyped so that efforts to utilize molecular tools to map quantitative trait loci within breeding populations can be pursued for crop improvement.

The National Clonal Germplasm Repository (NCGR) of the United States Department of Agriculture - Agricultural Research Service (USDA-ARS) in Davis, CA, has conserved about 200 genotypes of pomegranate in their Winters, CA experimental orchard. Many of them have consumer-friendly phenotypic characteristics, not perceived in the industry standard cultivar 'Wonderful' (Stover and Mercure, 2007). Previous investigations from our laboratories have demonstrated differences in morphology and vegetative growth traits during the propagation phase of the present study (Chater et al., 2017). Studies have shown that there can be differences among cultivars for many agriculturally-relevant physiological traits of pomegranate in other collections, including transpiration rate, stomatal conductance, water-use efficiency, photosynthetic rate and chlorophyll content (Drogoudi et al., 2012; Hepaksoy et al., 2000). The objectives of this research were: 1) to evaluate eleven unique pomegranate cultivars for field performance in semi-arid and coastal Mediterranean climates to determine plant establishment rates and site effects on reproductive biology; and 2) to evaluate four pomegranate cultivars for field performance in coastal versus inland agroecosystems to determine if there are differences among the genotypes for physiological traits that would be conducive to commercial crop production in drought conditions.

2. Materials and methods

2.1. Site conditions

The trials were conducted at the Agricultural Experiment Station of the University of California, Riverside, CA (33° 58′ 9.39″ N, 117° 20′ 46.93″ W, approximate elevation 340 m above the sea level) and on private land in Somis, CA (in proximity to 34° 15′ 26″ N 118° 59′ 43″ W, approximate elevation 98 m above the sea level) (Table 1). Riverside, CA (Riverside) is a semi-arid climate with hot, dry summers and cool winters whereas Somis, CA (Somis) is a coastal Mediterranean climate with warm, dry summers and wet, cool winters. Climate descriptions are based on Köppen climate classification. At Riverside, the mean annual precipitation of the area is 262 mm and mean high temperatures are 28 °C and 36 °C for June and August, respectively. Mean temperature lows are 13 °C and 18 °C for June and August, respectively and 5 °C, 6 °C, and 7 °C for December, January and February, respectively. The soil is a sandy loam with good drainage and was previously an established lemon grove. At Somis, mean annual precipitation of the area is 468 mm and mean high temperatures are 26 °C and 28 °C for June and August, respectively. Mean temperature lows are 13 °C and 14 °C for June and August, respectively and 5 °C, 5 °C, and 6 °C for December, January and February, respectively. The soil at Somis is a heavy sandy clay that was previously an established avocado grove. All trees were growing outside in field conditions with drip irrigation three times per week to replace water lost to soil evapotranspiration (ETo) as determined by California Irrigation Management Information System (CIMIS) weather stations in Riverside and Ventura counties. Trees were grown under conventional commercial management practices and fertilized in spring with urea and potassium sulfate, totaling 31.75 kg N, and 34 kg K per year, respectively, over approximately 0.81 ha (Approximately 39 kg/ha N and 42 kg/ha K). All experimental trees were followed during their first four years of development and were located on the inside of the grove, with at least one border tree acting as a buffer to reduce the edge effect.

2.2. Plant material

Two pomegranate cultivar trials were utilized for this study. The cultivars selected for the study were Ambrosia, Desertnyi, Eversweet, Golden Globe, Green Globe, Haku Botan, Loffani, Parfianka, Phoenicia, cv. 857 (an heirloom cultivar from Ventura County, USA) and Wonderful (Table 2). Cultivars were either cultivars bred for coastal sites or those that are typically grown in inland sites. Fruit acidity was the basis used for coastal versus inland cultivar categorization, with low acid cultivars considered for coastal and medium to high acid cultivars considered for inland applications. Coastal cultivars included Ambrosia, Eversweet, Golden Globe, Green Globe, and Loffani. Inland cultivars included Desertnyi, Haku Botan, Parfianka, Phoenicia, and Wonderful. For the heirloom cultivar included (cv. 857), it is unknown whether it is a coastal or inland cultivar. All genotypes were sourced from the National Clonal Germplasm Repository, Davis, CA, and propagated via dormant hardwood cutting at the same time in winter of 2012. All trees included were mature and most cultivars were bearing commercial loads of fruit by the fourth year. For physiological trait evaluations, three trees per cultivar were selected from a cultivar trial that consisted of a randomized complete block design with five blocks, three trees per cultivar per block, for a total of 15 trees per cultivar in the entire orchard. One tree was selected from three of the five blocks of the trial each day of measurement. Wonderful is the industry standard in many countries and was chosen as a control in the cultivar field trial. Wonderful is a widely-grown commercial cultivar that originated in Florida, it accounts for approximately 90–95% of production in the USA. It is a vigorous, thorny tree that has high yield with red fruit that have red arils with moderate seed hardness and a sweet-tart flavor. The growth habit is willowy, with a tendency to sucker at the base of the tree. There exists limited quantitative field establishment or physiology data for Wonderful and all other cultivars in this study.

2.3. Establishment measurements

Trunk diameter was measured in cm with a digital caliper in spring of each year, with the measurement taken approximately 15 cm above the soil level. Tree canopy diameter was measured in-row and between rows to determine how quickly trees were growing into each other and into the rows. Tree height and in- and between row spacing measurements were taken with a folding rule.

Equation 2.1 Equation for area of canopy.

$$S = \pi bx^2 + \frac{\pi ab}{e}\sin^{-1}e$$

Equation 2.2 Equation for eccentricity of ellipse.

Table 1
Site conditions after three years of growth of two cultivar field trials in Riverside, CA and Somis, CA planted with pomegranate cultivars sourced from the USDA-ARS National Clonal Germplasm Repository. Variables include soil mineral nutrient elements N, P, K (exchangeable), Ca (exchangeable), Mg (exchangeable), Na (exchangeable), S, B, Cu, Fe, Mn, Zn (in $g{\cdot}m^{-3}$). All mineral nutrients are reported in $g{\cdot}m^{-3}$. Soil analyses were conducted by a private laboratory.

Site	N	P	K	Ca	Mg	Na	S	B	Cu	Fe	Mn	Zn
Riverside, CA	12.7	13.6	240.5	4265.7	1338.6	99.3	86.8	0.4	1.5	24.0	22.8	10.0
Somis, CA	32.7	10.0	231.3	4228.9	1301.8	147.1	251.5	0.3	1.2	22.1	8.53	3.09

Table 2
Descriptions of the ten pomegranate cultivars sourced from the USDA-ARS National Clonal Germplasm Repository. Information known includes country of origin, countries of commercial production, acidity, flavor, peel color, aril color, and seed hardness.

Cultivar	Country of origin	Countries of commercial production	Acidity	Flavor	Peel color	Aril color	Seed hardness
Ambrosia	USA (Coastal)	USA	Low-medium	Sweet	Pink and red	Pink-red	Medium hard
Desertnyi	Turkmenistan	None	Medium-high	Sweet-tart	Red and pink	Red	Soft
Eversweet	USA (Coastal)	USA	Very Low	Sweet	Pink and yellow	Pink	Soft
Golden Globe	USA (Coastal)	None	Low	Sweet	Yellow and pink	Pink	Hard
Green Globe	USA (Coastal)	None	Low	Sweet	Yellow, green and pink	Pink	Hard
Haku Botan	Japan	Japan, USA (ornamental)	Very High	Sour	White/yellow	White	Hard
Loffani	USA (Coastal)	None	Low	Sweet	Yellow with pink	Pink	Hard
Parfianka	Turkmenistan	USA, Australia	Medium-High	Sweet-tart	Red	Red	Soft
Phoenicia	USA (coastal)	None	High	Sweet-tart	Yellow and pink	Red	Medium hard
cv. 857	USA (coastal)	None	Medium	Sweet-tart	Red	Dark red	Medium
Wonderful	Florida (Inland)	USA, Chile, Peru, Israel, Mexico, Argentina, South Africa, Uruguay, Turkey, Italy, Spain, Greece	Medium-high	Sweet-tart	Red	Red	Medium hard

$$e = \sqrt{1-\left(\frac{b}{a}\right)^2}$$

Tree canopy area was roughly approximated with an equation and associated method reported by Serfontein and Catling (1968), where "S" is canopy area, "a" is tree height, "b" is skirt radius and "e" is eccentricity of an ellipse.

Relative leaf chlorophyll content of three leaves per plant was measured with a SPAD-502 chlorophyll meter (Minolta, Japan).

2.4. Photosynthesis measurements

The four cultivars chosen for photosynthesis measurements were Eversweet, Haku Botan, Parfianka and Wonderful because of their variation in phenotype. Only four cultivars were chosen because of the time it takes to collect this photosynthesis data with the infrared gas analyzer used in this investigation. During fruit development (late July through September), an infrared gas analyzer (6400, LI-COR, Lincoln, NE, USA) was used to measure maximum rates of net CO_2 assimilation (A), stomatal conductance (g_s), and transpiration (E) during the morning (9 a.m.–12 p.m.). Morning light availability ranged from 1500–1600 μmol m^{-2} s^{-1} photosynthetic photon flux density (PFD). Photosynthesis measurements for three days were pooled for the four cultivars, which occurred on 29 July 2016, 4 August 2016, and 7 September 2016 for Riverside and 25 July 2016, 31 July 2016, and 31 August 2016 for Somis. Two leaves per tree and a minimum of three trees per cultivar were analyzed with the infrared gas analyzer. All leaves were collected, and leaf area was quantified with a leaf area meter to normalize the photosynthesis data (Li-Cor, Lincoln, NE, USA). Only the most recently fully-formed, sun-exposed leaves were selected for photosynthesis measurements in this investigation. Cuvette temperatures were allowed to vary with field conditions. Leaves were measured in a chamber for leaf photosynthesis measurements that provided 1500 μmol m^{-2} s^{-1} photosynthetic photon flux density (PFD). Instantaneous water-use efficiency was calculated as A/E and intrinsic water-use efficiency was calculated as A/g_s. The difference between these two traits is that intrinsic water-use efficiency corrects for differences in vapor pressure deficit whereas instantaneous water-use efficiency does not. This is important because knowing if climate affects water-use efficiency is useful for cultivar selection.

2.5. Stem water potential measurements

Predawn and midday stem water potential measurements were taken for each day of photosynthesis data collection. The three trees selected were from the blocks chosen for the study. For both predawn and midday water potential, non-actively growing shoots were covered with a plastic bag for 10 min before being pruned, placed in a sealed plastic bag and kept in a cooler bag until transferred to a cool, lit indoor environment for balancing pressure measurements with a pressure chamber (Model 1000 Pressure Chamber, PMS Instrument Company, Albany, USA). For afternoon stem water potential measurements, canopy-shaded non-actively growing shoots were covered with a plastic bag for 10 min before being pruned.

2.6. Precocity measurements

Flowers were counted during peak bloom, defined as 90% or more of the flowers being open in the grove, which was typically in April and early May of each year (2014–2017). Male flowers, as identified by their signature bell shape in pomegranates, were counted along with the female flowers, which were identified by their easily recognizable vase shape. For reasons unknown, 'Haku Botan' flowers had morphological differences between sites that made determining male and female difficult at Somis, the coastal site. Male to female flower ratio was calculated by dividing number of male flowers by the number of female flowers. Total number of flowers was determined by adding male flowers with female flowers. Number of fruit was determined by counting fruit after fruit set in the months of July and August.

2.7. Statistical analysis

All variables were analyzed with Analysis of Variance (ANOVA). When ANOVA indicated significant differences, post-hoc comparisons were performed utilizing Tukey's honestly significant difference (HSD) test with an experiment wise error rate of $\alpha = 0.05$. Relationships between all variables were analyzed using linear regression ($\alpha = 0.05$), with relationships between variables determined using general regression with Minitab Software, version 16 (Coventry, UK). For the purposes of this work, the R value is the percentage of variation in the dependent variable that is explained by the variation in the independent variable. Regression models were fit to determine differences in slope coefficients and constants (y-intercept) among variables using regression analysis.

3. Results

3.1. Establishment measurements

Trees at both sites established at varying rates, with low tree mortality for some cultivars and zero tree mortality for others. After three years of growth, trunk diameter was significantly different between sites ($P < 0.001$), with a mean trunk diameter of 7.6 cm in Riverside and 6.4 cm in Somis. Every year, trunk diameter was larger at Riverside

Table 3
Orchard establishment at the end three years of growth of ten pomegranate cultivars sourced from the USDA-ARS National Clonal Germplasm Repository. Traits evaluated included trunk diameter (cm), canopy area (m^2), and relative chlorophyll content (SPAD units). All experimental trees were in their third year of growth in field trials in Riverside, CA, and Somis, CA.

Cultivar	Trunk diameter		Canopy area		Relative chlorophyll content	
	Riverside	Somis	Riverside	Somis	Riverside	Somis
Ambrosia	6.9[1]	7.7a[2]	43.1ab	42.8a	36.2ab	39.2b
Desertnyi	6.0	4.4c	23.4ef	22.9c	35.0ab	38.6b
Eversweet	7.1	5.0bc	17.6f	23.7bc	35.6ab	37.0b
Golden Globe	8.4	7.0ab	29.3cde	37.9abc	39.4ab	43.1b
Green Globe	8.5	8.8a	53.3abcde	40.9abc	35.5ab	44.2ab
Haku Botan	8.1	7.4a	30.1bcde	42.8a	50.6a	57.5a
Loffani	7.5	6.0abc	25.4def	37.8abc	25.0b	41.8b
Parfianka	7.0	5.8abc	37.4abc	39.3ab	36.6ab	42.6b
Phoenicia	7.6	6.3abc	39.5abc	36.9abc	44.9ab	40.6b
cv. 857	9.1	7.6a	43.7a	41.3a	51.9a	44.6ab
Wonderful	7.7	7.1ab	35.4abcd	43.7ab	45.1ab	44.3b
Overall means	7.6	6.4	34.1	37.4	40.2	42.4
P-value	NS[3]	< 0.001	< 0.001	< 0.001	0.010	< 0.001

[1] Values expressed in means.
[2] Values followed by different letters within a column are significantly different ($P < 0.05$).
[3] NS = Not significant.

compared to Somis. There were significant differences among cultivars for trunk diameter at Somis, but not at Riverside (Table 3). 'Desertnyi' had a relatively thin trunk at Somis, which was significantly thinner than 'Wonderful.' During fruit development (June through October), Riverside and Somis had 36,998 and 36,180 W/m^2 of total solar irradiance, respectively.

Canopy area was larger at Somis ($P = 0.039$), with Riverside having 34.1 m^2 canopy area on average and Somis having an average of 37.4 m^2 canopy area (Table 3). There were significant differences among cultivars at each site (Table 3). At Riverside, Desertnyi, Eversweet, and Loffani had grown slowest compared to the other cultivars. At Somis, four cultivars grew at a significantly higher rate than the others. The faster establishing cultivars at the coastal site were Ambrosia, Haku Botan, cv. 857, and Wonderful. Of the remaining cultivars at Somis, Desertnyi, Eversweet and Golden Globe were the slowest growing cultivars during establishment. Results for in-row and between-row establishment were similar in Somis, where Ambrosia, Green Globe, Haku Botan, cv. 857, and Wonderful had the fastest establishment (Table 4).

There were significant differences between sites and among cultivars for relative chlorophyll content. Pomegranate plants grown at the Riverside location had lower leaf relative chlorophyll content than those grown at Somis ($P < 0.001$), with average values of 40.2 and 42.4 SPADs for Riverside and Somis, respectively. 'Haku Botan' had the highest relative chlorophyll at Somis and 'cv. 857' had the highest relative chlorophyll content at Riverside (Table 3). Haku Botan had significantly higher SPAD values than Loffani at Riverside and had significantly higher SPAD values than all cultivars except for Green Globe and cv. 857 at Somis. Of all cultivars at both sites, Loffani had notably low relative chlorophyll content at Riverside.

There were significant differences among cultivars and between sites for tree height (Table 4). Somis had the tallest trees ($P < 0.001$), and had mean tree height 231.4 cm compared to 182.7 cm at Riverside. The tallest cultivars at Riverside were Haku Botan, Phoenicia, cv. 857, and Wonderful, which were significantly taller than Desertnyi and Eversweet. At Somis, Ambrosia, Golden Globe, Haku Botan and cv. 857 were the tallest, and these cultivars were significantly taller than Desertnyi and Eversweet.

Results for in-row and between row canopy diameter were similar to those of canopy area. Trees at Somis established and grew larger than trees at Riverside (Table 4). Values for in-row and between row canopy diameter were similar for a given cultivar, demonstrating uniformity of in-row and between-row growth. There were also significant differences among cultivars at each site, with Desertnyi and Eversweet smaller than most other cultivars at both sites.

Table 4
Orchard establishment of ten pomegranate cultivars sourced from the USDA-ARS National Clonal Germplasm Repository. Traits evaluated included tree height (cm), in-row canopy diameter (cm), and between-row canopy diameter (cm). All experimental trees were in their fourth year of growth in field trials in Riverside, CA, and Somis, CA.

Cultivar	Tree height		In-row canopy diameter		Between-row canopy diameter	
	Riverside	Somis	Riverside	Somis	Riverside	Somis
Ambrosia	176.8abcd[1,2]	240.4a	186.1cdef	255.4a	181.9bcd	261.9ab
Desertnyi	149.0cd	143.9c	173.7ef	143.3d	180.0cd	151.0d
Eversweet	144.3d	165.4bc	157.1f	162.2cd	168.5d	168.0d
Golden Globe	183.9abc	237.9a	189.0bdef	185.3bcd	192.9abcd	198.5cd
Green Globe	206.4abcd	249.9ab	228.6ab	262.1ab	209.6abcd	303.3a
Haku Botan	213.7a	238.4a	177.8def	188.3abcd	190.8abcd	201.2bcd
Loffani	155.1bcd	214.0ab	176.3def	190.3bcd	181.9cd	205.2bcd
Parfianka	186.9abc	199.3abc	216.2abcde	207.9abc	208.6abc	209.9bcd
Phoenicia	211.2a	209.3ab	219.8abcd	198.5abcd	223.0ab	198.1cd
cv. 857	190.2ab	256.8a	237.4ac	248.4ab	224.5a	253.0abc
Wonderful	190.7ab	223.3ab	211.1abcde	240.4ab	206.3abc	244.0abc
Overall means	182.7	213.4	195.1	203.0	195.5	211.0
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

[1] Values expressed in means.
[2] Values followed by different letters within a column are significantly different ($P < 0.05$).

Table 5
Physiological traits of four pomegranate cultivars sourced from the USDA-ARS National Clonal Germplasm Repository. Mean values of maximum rates of net CO_2 assimilation (μmol CO_2 $m^{-2} s^{-1}$, *A*), stomatal conductance (mol H_2O $m^{-2} s^{-1}$, g_s), transpiration (mmol H_2O $m^{-2} s^{-1}$, *E*), intrinsic water-use efficiency ($A{\cdot}g_s^{-1}$), instantaneous water-use efficiency ($A{\cdot}E^{-1}$) for four pomegranate cultivars. Measurements were made in the morning hours during fruit development in summer of 2016. Experimental trees were in their fourth year of growth in field trials in Riverside, CA, and Somis, CA.

Cultivar	*A*		g_s		*E*		$A{\cdot}g^{-1}$		$A{\cdot}E^{-1}$	
	Riverside	Somis	Riverside	Somis	Riverside	Somis	Riverside	Somis	Riverside	Somis
Eversweet	15.30ab[1,2]	11.55	0.096ab	0.091	2.311ab	1.621	171.0	151.7	7.05	5.60
Haku Botan	12.77b	13.09	0.085b	0.130	1.978b	1.804	165.9	131.8	7.09	8.59
Parfianka	14.40ab	13.34	0.108ab	0.084	2.374ab	1.537	145.4	169.3	6.51	9.21
Wonderful	17.65a	12.73	0.137a	0.090	3.151a	1.495	132.1	153.1	6.14	9.97
Overall means	15.03	12.68	0.107	0.097	2.45	1.61	153.6	151.5	6.70	8.99
P-value	0.045	0.590	0.034	0.386	0.045	0.812	0.254	0.188	0.781	0.330

[1] Values expressed in means.
[2] Values followed by different letters within a column are significantly different ($P < 0.05$).

3.2. Physiology measurements

The four pomegranate cultivars chosen for photosynthesis measurements were actively photosynthesizing and transpiring during morning hours at both sites during all days of data collection (Table 5). There were significant differences among cultivars for photosynthesis (*A*) at Riverside but not at Somis. 'Haku Botan' had significantly lower rates of *A* ($P = 0.045$), g_s ($P = 0.034$) and *E* ($P = 0.045$) than 'Wonderful' during the morning hours. There were no other differences detected for photosynthetic variables among the four cultivars investigated.

The effect of site on pomegranate physiology was significant. Riverside had significantly higher rates of photosynthesis (*A*) than Somis, with mean 15.0 and 12.7 μmol CO_2 $m^{-2} s^{-1}$ for Riverside and Somis, respectively (Table 5). For *A*, rates at Riverside were about 15% higher on average than Somis ($P < 0.003$). Rates of g_s at the two sites were similar, with Riverside and Somis having 0.107 and 0.097 mol H_2O $m^{-2} s^{-1}$, respectively. Rates of *E* were about 34% higher on average at Riverside than at Somis ($P < 0.001$). Mean rates of *E* were 2.45 mmol H_2O $m^{-2} s^{-1}$ for Riverside and 1.61 mmol H_2O $m^{-2} s^{-1}$ for Somis. There was no difference in intrinsic water-use efficiency among cultivars or between Riverside and Somis. There were significant differences between sites for instantaneous water-use efficiency, with Somis having significantly higher instantaneous water-use efficiency than Riverside ($P = 0.001$). 'Wonderful' had significantly higher rates of $A{\cdot}E^{-1}$ at Riverside than at Somis, and 'Wonderful' had significantly higher rates of $A{\cdot}E^{-1}$ than "Eversweet" at Somis and "Haku Botan" at Riverside ($P = 0.001$).

There were no significant differences among cultivars or between sites for predawn stem water potential (Fig. 1). Predawn stem water potential was significantly less negative than midday stem water potential ($P < 0.001$). There were significant differences between sites and among cultivars for midday stem water potential (Fig. 1). Somis had less negative midday stem water potentials than Riverside (UCR) ($P < 0.001$), with Riverside having a mean stem water potential of −2.31 MPa and Somis having a mean stem water potential of −1.53 MPa for midday measurements. 'Haku Botan' had less negative stem water potential than 'Parfianka' at Riverside ($P = 0.009$) (Fig. 1). There were no differences in stem water potential among cultivars for midday measurements at Somis.

There were positive and negative correlations between physiological variables for morning, afternoon and for data pooled for the two times of day. The relationship between *A* and g_s was positively correlated and statistically significant for photosynthesis measurements for pooled data from all cultivars combining both sites ($R = 0.2896$, $P < 0.001$) at Riverside ($R = 0.2896$, $P < 0.001$) and at Somis ($R = 0.2108$, $P < 0.005$). There was also a positive correlation between *A* and *E* for pooled data from both sites ($R = 0.1872$, $P = 0.008$), at Riverside ($R = 0.4186$, $P < 0.001$) and at Somis ($R = 0.2456$, $P = 0.002$).

3.3. Precocity measurements

There were site differences for precocity (Table 6). Riverside had significantly higher precocity than Somis ($P < 0.001$), with Riverside having 138.6 flowers on average and Somis having 28.7 flowers on average by year four. For each cultivar at both sites, number of male flowers was greater than number of female flowers (Table 6). There

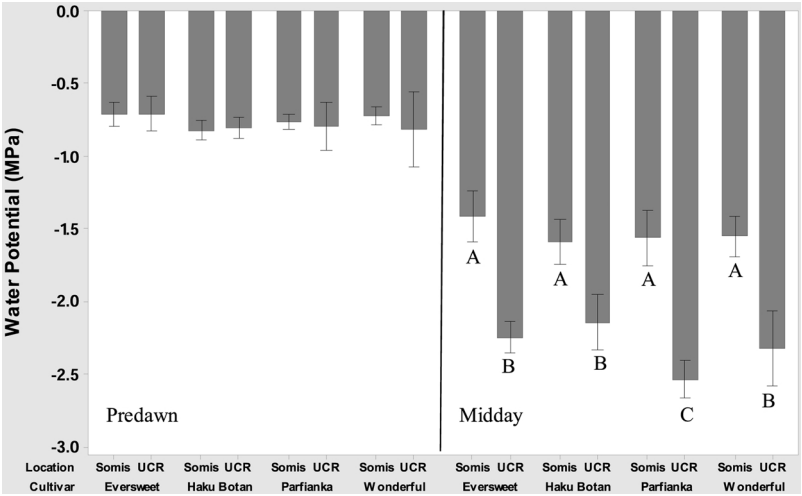

Fig. 1. Mean stem predawn and midday water potential (MPa) of four pomegranate cultivars grown in field trials in Riverside, CA (UCR) and Somis, CA, USA (Somis). All stem water potential measurements were made in the predawn or afternoon hours during fruit development in summer of 2015 and 2016. Values followed by different letters within a column are significantly different ($P < 0.05$; n = 9, data were pooled for each cultivar-site combination).

Table 6
Reproductive traits of ten pomegranate cultivars sourced from the USDA-ARS National Clonal Germplasm Repository. Mean values of number of male flowers, number of female flowers, male to female flower ratio, total number of flowers, and number of fruit. All experimental trees were in their third year of growth in field trials in Riverside, CA, and Somis, CA.

Cultivar	No. male flowers		No. female flowers		Male:Female ratio		Total no. flowers		No. Fruit	
	Riverside	Somis	Riverside	Somis	Riverside	Somis	Riverside	Somis	Riverside	Somis
Ambrosia	104.7abc[1,2]	8.7c	39.3abc	5.5b	2.7	2.0	144.0abc	14.1c	23.3cd	2.3bc
Desertnyi	56.7bc	10.7c	20.8bc	7.5b	8.5	3.5	77.4bc	17.0c	21.7cd	4.4bc
Eversweet	34.2c	8.0c	24.6bc	4.4b	1.1	1.1	58.7c	12.4c	11.0de	0.3c
Golden Globe	126.8abc	9.1c	31.5abc	6.5b	3.6	1.9	158.3abc	15.6c	20.8cde	0.7c
Green Globe	258.8ab	101.3a	37.8abc	25.8ab	17.0	4.4	296.5ab	127.0a	34.0bc	11.0ab
Haku Botan	41.0bc	n.d.	13.7c	n.d.	2.1	n.d	54.7.9bc	25.2bc	10.1de	1.7bc
Loffani	59.8bc	0.6c	11.7c	0.2b	8.8	3.5	71.4cd	0.8c	2.5e	0.1c
Parfianka	94.2abc	25.0bc	31.2abc	7.8b	3.1	3.5	125.4abc	32.8bc	44.9b	6.4abc
Phoenicia	166.2a	22.8bc	37.6abc	16.5ab	6.0	1.7	203.8a	39.3bc	26.7cd	2.7bc
cv. 857	80.6abc	17.8bc	61.0a	11.7ab	1.3	1.3	141.6abc	29.5bc	68.0a	5.3bc
Wonderful	143.ab	50.4ab	48.8ab	26.5a	3.1	1.7	161.1abc	72.1ab	52.3ab	12.6a
Overall means	95.2	19.6	30.0	10.4	4.8	2.2	114.2	28.7	n.d.[3]	n.d.
P-value	< 0.001	< 0.001	< 0.001	< 0.001	0.259	0.265	< 0.001	< 0.001	< 0.001	< 0.001

[1] Values expressed in means.
[2] Values followed by different letters within a column are significantly different ($P < 0.05$).
[3] n.d. = not determined.

were significant differences among cultivars at both sites for number of male flowers and number of female flowers. However, there was no evidence for significant differences among cultivars at either site for male:female flower ratio. There were also significant differences among cultivars at both sites for total number of flowers and total number of fruit. Riverside had significantly more fruit than Somis in the first three years, but not for year four. For unknown reasons, 'Green Globe' had a much higher male:female flower ratio at Riverside compared to Somis. This site effect can be due to climatic effects, with factors such as spring and summer cloud cover, chilling hours, and temperature possibly causing differences between sites for flower production. Soil differences in nutrition or texture could also play a factor, but these hypotheses would have to be investigated further to know one way or the other.

This investigation provided evidence that some pomegranate cultivars are more productive than others during the first four years of establishment. In Somis, the most precocious and productive cultivars in this investigation were Green Globe and Wonderful. 'Wonderful' was rather productive at both sites. Haku Botan was the only ornamental "double flower" cultivar in the trial and its flowers were difficult to sex at Somis. Observationally, 'Haku Botan' had larger flowers at the Somis, the coastal site. This phenotype made it difficult to sex the flowers and thus only total number of flowers was determined for this cultivar at that site. Usually, pomegranate flowers are easy to sex based on their shape, with bell-shaped flowers being males and vase-shaped flowers being female. This distinction was not possible for 'Haku Botan' at Somis during the four years of study.

4. Discussion

The objectives of this research were to evaluate eleven unique pomegranate cultivars for field performance in semi-arid and coastal Mediterranean climates, to determine plant performance and site effects on reproductive biology, and to evaluate four pomegranate cultivars in coastal versus inland agroecosystems to assess whether there were differences among genotypes for physiological traits that would be conducive to commercial crop production in drought conditions. The purpose was to phenotype these accessions for grower suitability and to aide in future breeding endeavors for crop improvement and germplasm diversity enhancement. Our findings indicate that many of the cultivars established similarly to Wonderful, but not all. Namely, 'Desertnyi' and 'Eversweet' grew less at both sites. 'Eversweet' was known to be less vigorous among rare fruit cognoscenti and 'Desertnyi' has been known to have trouble with canopy architecture at the germplasm repository (personal communication). Our findings provide evidence that these two cultivars may not be the best choices to grow in conventional spacing for the Californian pomegranate cropping system, unless perhaps rootstocks are employed that alter their growth rate. Literature on rootstocks is limited, so more research is needed to determine if these two cultivars would benefit from being grafted on a more vigorous rootstock (Vazifeshenas et al., 2009). If these cultivars were to be chosen for commercial production due to their soft seeded arils and ornamental fruit, a possible method for commercial production would be to reduce spacing to 2–3 m and/or to grow them in a trellis system.

For at least one of the two sites, All four cultivars evaluated for physiological traits in these cultivar studies displayed eco-physiological traits suitable for commercial production if the industry standard, Wonderful, is used as the standard for comparison. However, 'Wonderful' performed better at Riverside than at Somis for *A*, which may indicate that 'Wonderful' may be better planted for commercial production inland than in coastal regions. Photosynthesis values obtained for 'Wonderful' at Riverside were different than those reported in Greece (Noitsakis et al., 2016), but similar enough to suggest the possibility that differences in climate or cultural practices between the two sites were the source of this discrepancy. Noitsakis et al. (2016) reported photosynthetic rate values approximately 12.5 to 14 μmol CO_2 $m^{-2} s^{-1}$ for their control irrigation treatment of 'Wonderful', which is slightly different from the 17.7 μmol CO_2 $m^{-2} s^{-1}$ on average for Riverside 'Wonderful', but consistent with 'Wonderful' at Somis, which was found to have a photosynthetic rate of 12.7 μmol CO_2 $m^{-2} s^{-1}$ on average. We found evidence that there are differences among cultivars within a site for physiological traits including stomatal conductance, transpiration and midday water potential. Values for physiological traits were similar to previous investigations into pomegranate physiology (Hepaksoy et al., 2000; Rodríguez et al., 2012), a topic which is limited in the literature. Rodríguez et al. (2012) reported stomatal conductance values between approximately 120 and 450 mmol H_2O $m^{-2} s^{-1}$, which were values typically higher than recorded at both sites in our study, which were 137 and 90 mmol H_2O $m^{-2} s^{-1}$ on average for Riverside and Somis, respectively. For midday stem water potential, Rodríguez et al. (2012) reported a range of −2.0–−0.7 MPa, which is similar to Somis midday stem water potential values of −1.5– − 1.7, but much less negative than Riverside midday stem water potential values, which ranged from −2.2–−2.7 MPa. Differences were also detected between sites, with higher rates of assimilation and transpiration at Riverside compared to Somis. There were no differences in intrinsic water-use efficiency between sites. There was a significant difference between sites for instantaneous water-use efficiency, with

Somis having more efficient water use than Riverside. This indicates that some pomegranate cultivars may use less water for commercial production on the coast, but more studies must be done on irrigation scheduling to determine if these cultivars can be grown with less water on the warm coast of Southern California compared to hotter, semi-arid climates inland. Less irrigation was applied at Somis due to lower ETo at that site. Soil ETo·yr^{-1} at Riverside and Somis were 1194 mm and 895.7 mm on average.

There were also differences among cultivars for photosynthetic assimilation, stomatal conductance, and transpiration at Riverside, but not at Somis, with 'Haku Botan' having significantly lower rates of assimilation, stomatal conductance and transpiration than 'Wonderful' at Riverside. No other differences for these physiological traits were detected. Because the main differences observed were in Riverside, and included g_s and E which describe water loss, examining water loss characteristics offers a promising direction for improving water-use efficiency during cultivar selection.

The most interesting findings of this field study were that there were differences among cultivars and between sites for establishment rates, important physiological traits, such as A, E, g_s, water potential, and precocity. These findings provide a glimpse into phenotypes of USDA-ARS NCGR cultivars and suggest there may be other cultivars in the national germplasm, or in other germplasm collections, that have even faster establishment rates, greater production efficiencies, and higher precocity than those represented in this study. These findings are important not only for growers looking for cultivars that use less water, grow faster and have higher yields with less irrigation for higher profits and more sustainable agricultural practices, but also for breeders who can use this information to make informed decisions regarding genotype selection for crosses. Hepaksoy et al. (2000) reported evidence that E and instantaneous water-use efficiency of pomegranate may be correlated with fruit split in some cultivars, which means that those cultivars demonstrating differences for these physiological traits should be followed in the field to determine their effects on fruit split. The next step in this discovery of differences in physiological traits is to investigate what other factors determine why one cultivar is more efficient with water than another.

Literature regarding physiology of pomegranate trees is limited. The results of the present investigation are similar to investigations into physiological differences among pomegranate cultivars in other germplasm collections (Drogoudi et al., 2012). Because information regarding precocity in pomegranate germplasm is limited, this study provides some insight to pomegranate reproductive biology in an experimental field trial setting. Water potential values agree with other investigations which included midday stem water potential (Hepaksoy et al., 2000; Rodríguez et al., 2012), but data for predawn water potential are scant in the literature. These are the first known values for water potential in predawn hours for California-grown pomegranate. At Riverside, there were significant differences among cultivars for midday water potential, but not for predawn water potential. The low midday stem water potentials provide evidence that these trees were under water stress in the hot summer conditions of Riverside. These findings indicate that we are seeing differences in water uptake during the middle of the day, and that trees are rehydrated at night.

We studied eleven unique pomegranate cultivars displaying very different phenotypes and found some interesting differences among them in terms of establishment rate, physiological traits, and precocity. Despite these differences, many of the cultivars investigated were determined to perform virtually the same as Wonderful in semi-arid and coastal climates, though not all. As we found differences among cultivars for various physiological traits relevant to water-use efficiency, the next steps would be to conduct more studies with more than four cultivars, and to also carry out these physiological and water relations measurements during different times of the year and at different times of the day. It would also be important to investigate these cultivars on molecular, morphological or anatomical scales to determine the underlying causes of these differences among cultivars for breeding purposes.

5. Conclusions

This study was the first of its kind in evaluating establishment rates, physiological traits and precocity in California-grown Wonderful and other pomegranate cultivars in the USDA-ARS national clonal germplasm collection. These results further our understanding of how unique pomegranate trees from the national germplasm collection function on vegetative, physiological, and reproductive levels while being established at two sites with different climates. This study suggests that water-use efficiency can be improved through cultivar selection and that some cultivars may be better suited for reduced tree spacing and/or trellis cropping systems than others. For example, 'Wonderful' had higher stomatal conductance and transpiration rates compared to 'Haku Botan'. For tree spacing practices, Desertnyi and Eversweet were shorter and occupied less space than other cultivars so they would possibly benefit from a planting with closer spacing and their thin trunks may benefit from plant support systems (e.g. trellising). We emphasize that the most precocious cultivars in this study are better suited to specific climates, and more studies are needed to determine regional cultivar suitability. More field trials should be planted to evaluate regional suitability of unique cultivars having consumer-friendly traits to determine their production efficiency and to identify those that are productive under high temperature conditions with less applied irrigation water.

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